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Metabolic effects of 4-pentenoate on isolated dog kidney tubules

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Metabolic effects of 4-pentenoate on isolated dog kidney tubules. The effects of 4-pentenoate (0.01 to 10 mM) were studied on suspensions of cortical tubules and of thick ascending limbs (TAL) prepared from dog kidneys. When cortical tubules were incubated with 1 mM glutamine, 4-pentenoate accelerated glutamine uptake, ammoniagenesis, and the production of alphaketoglutarate, lactate and pyruvate, but decreased gluconeogenesis. With 5 mM glutamine, the marked accumulation of alphaketoglutarate reversed the net fluxes through the alanine and aspartate aminotransferases. When cortical tubules or TAL were incubated with lactate, its utilization and gluconeogenesis (in cortical tubules) were markedly decreased by 4-pentenoate. The mitochondrial NAD+/NADH ratio was markedly increased by 4-pentenoate in cortical tubules but not in TAL. The production of $^{14}CO_2$ from ^{14}C[1]-pyruvate or ^{14}C-[1]-alphaketoglutarate was decreased by approximately 60% by 4-pentenoate in cortical tubules but not in TAL. In cortical tubules, these findings are best explained by depletion of mitochondrial free CoA, inhibition of pyruvate and alphaketoglutarate dehydrogenases and decreased mitochondrial NADH. By contrast, in TAL, accumulation of reducing equivalents probably resulted from the metabolism of 4-pentenoate itself.

We have shown in a recent in vivo study in the dog that the infusion of 4-pentenoate induced a complete Fanconi's syndrome with proximal renal tubular acidosis [1]. In this study, 4-pentenoate accelerated glutamine utilization and ammonia production and decreased lactate utilization and probably gluconeogenesis in the intact kidney. Previous in vitro studies have shown that 4-pentenoate, an unsaturated short-chain fatty acid, depletes the mitochondrial CoA pool through its sequestration into inert and stable derivative(s) of 4-pentenoate [2, 3]. The lack of free CoA then inhibits the CoA-dependent mitochondrial oxidation of fatty acids [4, 5], pyruvate [6, 7] and alphaketoglutarate [8] and the decreased acetyl CoA diminishes gluconeogenesis [5, 9–11]. The 4-pentenoate-induced increment in ammoniagenesis observed in our in vivo study [1] (as well as in rat renal cortical slices [10]) probably reflects a metabolic compensatory response to these effects of 4-pentenoate.

The present study was undertaken to throw some light on the sites and mechanisms of the metabolic response induced by 4-pentenoate along the nephron. Our study was carried out in dog tubules to remove extrarenal factors that might influence renal glutamine and lactate metabolism. In order to investigate the tissue specificity of the metabolic effects of 4-pentenoate, both proximal tubules and thick ascending limbs were studied. Our data demonstrate that in proximal tubules 4-pentenoate significantly accelerated renal glutamine metabolism (and ammonia production) to alphaketoglutarate which accumulated and markedly reduced the gluconeogenesis, the renal utilization of lactate and the fluxes through pyruvate and alphaketoglutarate dehydrogenases. In thick ascending limbs, a fall in the utilization of lactate was also induced by 4-pentenoate but no accumulation of alphaketoglutarate was noted and the fluxes through pyruvate and alphaketoglutarate dehydrogenases were not altered. It is concluded that the toxic effect of 4-pentenoate is mostly restricted to the proximal nephron.

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Methods

Experimental procedures

Metabolism of various substrates. Suspensions of cortical tubules (> 85% proximal) and of thick ascending limbs (> 95% purity) were prepared from the kidneys obtained from mongrel dogs. Following anesthesia with sodium pentobarbital (30 mg/kg), the dogs were intubated and connected to a Harvard respirator. Both kidneys were rapidly removed, immediately placed in ice-cold Krebs-Henseleit saline and cut into slices. The superficial cortex and the red inner stripe of the outer medulla were carefully dissected out with fine scissors. Each tissue fraction was then sliced with a Stadie-Riggs microtome and digested with collagenase during 45 minutes (cortex) or 60 minutes (red medulla) according to a procedure already described [12, 13]. Approximately 60 mg wet weight of renal cortical tubules or 30 to 40 mg wet weight of tubules of thick ascending limbs of Henle were incubated in a final volume of 4 ml of gassed (95% O_2 – 5% CO_2) Krebs-Henseleit saline containing 2.5 g % of dialyzed bovine albumin (fraction V) and adjusted to pH 7.4.

For each type of tubule, the incubation medium contained either 0, 0.01, 0.1, 1 or 10 mM 4-pentenoate, neutralized to a pH of 7.4. In six experiments performed with cortical tubules, the exogenous metabolic substrates also placed in the incubation medium were either 1 or 5 mM L-glutamine together with 0.1 or 0.5 mM L-glutamate or 1 or 5 mM L-lactate together with 0.1 or 0.5 mM pyruvate in order to obtain a physiological ratio. The substrate combination of glutamine and lactate was also studied. Four additional experiments were performed with 1 or 5 mM L-glutamate (with or without 1 or 5 mM L-lactate) and six

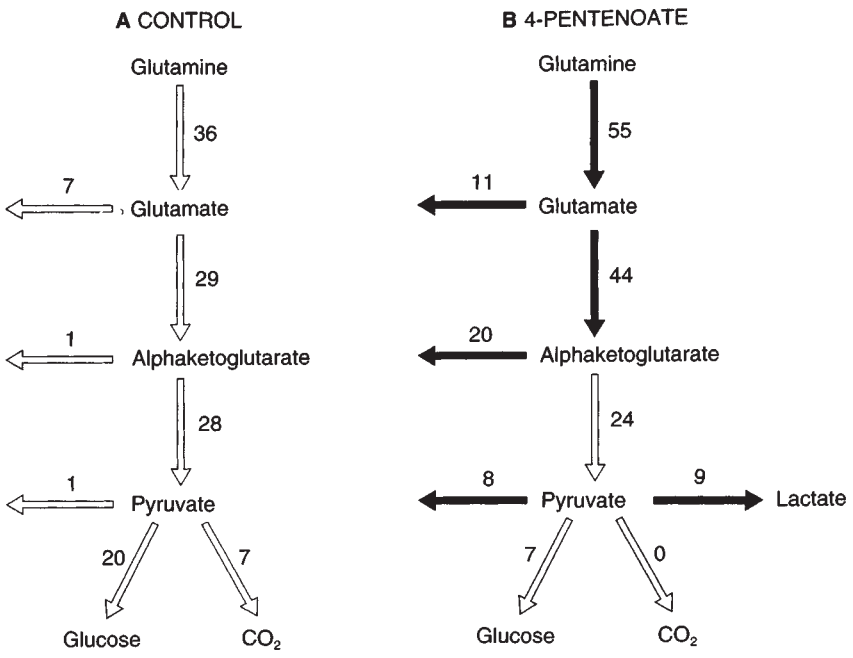

Fig. 1. *Renal cortical metabolism of glutamine 1 mM in the absence (A) and in the presence of 10 mM 4-pentenoate (B).* Means for glutamine extracted and glutamate, alphaketoglutarate, lactate, and pyruvate produced are given in Table 1; these values and the metabolic fluxes between these intermediates are expressed as micromoles per gram tissue wet weight per 60 minutes. The minimal utilization of aspartate and production of malate and alanine are not presented in this figure. Numbers close to each arrow represent mean fluxes calculated from measured values for reaction(s) represented by the arrow. Solid arrows mean fluxes accelerated by 4-pentenoate and open arrows decreases in these fluxes after 4-pentenoate. Various metabolites are shown to join pyruvate (oxaloacetate) pool, supporting gluconeogenesis and oxidation.

other studies were carried out with 1 or 5 mM malate or glycerol. In six experiments performed with thick ascending limbs, the substrates placed in the incubation medium were 1 or 5 mM lactate together with 0.1 or 0.5 mM pyruvate, respectively. In all cases the tubules were incubated in 25 ml siliconized erlenmeyer flasks for 0 or 60 minutes at 37°C with constant shaking (40 rpm) and the incubation was stopped by the addition of 40% perchloric acid (final concentration of 5%).

Oxygen consumption. In five additional experiments performed with 10 mM glutamine and 10 mM lactate in the medium, the effect of 1 mM 4-pentenoate on the oxygen consumption by the suspension of renal cortical tubules was measured polarographically using an oxygen electrode and a specially designed oxymetric chamber [14]. Four similar experiments were performed using a suspension of thick ascending limbs incubated with 10 mM lactate.

Metabolism of ^{14}C-[1]-pyruvate and ^{14}C-[1]-alphaketoglutarate. To study the production of $^{14}CO_2$ from the metabolism of ^{14}C-[1]-pyruvate or ^{14}C-[1]-alphaketoglutarate, tracer amounts (0.5 μCi/flask) of labelled pyruvate or alphaketoglutarate were added respectively to 2 mM cold pyruvate or alphaketoglutarate. For comparison purposes, five experiments were performed with dog cortical tubules or thick ascending limbs incubated in a final volume of 4 ml of Krebs-Henseleit saline. In each tissue, 2 mM substrates were studied with or without 1 mM 4-pentenoate.

Analytical methods

The acid supernatant was neutralized with potassium hydroxide and used to determine the concentrations of glutamine, glutamate, alphaketoglutarate, ammonia, malate, aspartate, alanine, lactate, pyruvate and glucose by enzymatic assays as previously reported [15]. Uptake or production of each metabolite was calculated using corresponding zero time values and expressed per gram wet weight and per 60 minutes of incubation (these processes having been previously demonstrated to be linear).

For experiments using ^{14}C-[1]-substrates, the incubation was performed at 37°C in flasks fitted with an empty center well fitted with a dry filter paper butterfly. The dead space was filled with 95% O_2:5% CO_2. At the end of the incubation (0, 15, 30, 60 minutes), the center well was filled up with 0.5 ml of 20% KOH and 0.25 ml of 40% perchloric acid was injected in the incubation medium with a long needle through the rubber cap closing the flasks. The incubation was continued at 4°C for two hours in order to collect all the CO_2 (gas + bicarbonate). The KOH and the filter paper were then collected and the well was washed twice with 1 ml of water. All the KOH and washing volumes were added in 15 ml of scintillation cocktail (Aquasol, Packard) in counting vials (a gel is formed under these conditions). The vials were left for 48 hours in the dark at 4°C and counted. A sample of the incubation medium was counted and the rest was deproteinized and neutralized as described above. The specific activity obtained at the beginning of the incubation was measured from the initial pyruvate (or alphaketoglutarate) concentration and DPM. The recovery of DPM as soluble metabolites + CO_2 was 100 ± 2.0% (N = 5).

Calculations

The metabolic fates (production of aminoacids or organic anions, gluconeogenesis, or oxidation to CO_2) of added substrates was estimated from a carbon balance [16]. When the sum of amino acids, organic anions and C_3-glucose was smaller than the total extraction of carbon substrates, the difference was assumed to represent net oxidation to CO_2. The apparent free NAD+/NADH ratio of the cytosolic and mitochondrial compartments were calculated respectively from the lactate

Table 1. Effects of 4-pentenoate on the metabolism of renal cortical tubules incubated with glutamine

4-Pentenoate concentration, mM	Glutamine	Glutamate	KG	Ammonia	Malate	Aspartate
Glutamine 1 mM						
0	−36.4 ± 3.3	7.1 ± 1.9	0.6 ± 0.2	84.7 ± 5.7	−0.1 ± 0.04	−2.0 ± 1.1
0.01	−37.0 ± 3.2	7.7 ± 2.3	0.6 ± 0.2	92.3 ± 4.9	−0.1 ± 0.1	−2.1 ± 1.0
0.1	−35.3 ± 3.8	9.7 ± 2.5	0.5 ± 0.2	85.4 ± 7.2	−0.1 ± 0.1	−1.6 ± 1.1
1	−50.2 ± 4.2[b]	11.9 ± 1.7[a]	15.5 ± 0.9[b]	99.6 ± 5.9[a]	0.6 ± 0.3	−2.8 ± 1.1
10	−55.0 ± 4.4[b]	11.1 ± 1.9	20.2 ± 1.3[b]	109.5 ± 7.2[b]	0.9 ± 0.5[a]	−3.4 ± 0.8[a]
Glutamine 5 mM						
0	−110.5 ± 17.8	41.2 ± 3.8	1.4 ± 0.3	190.7 ± 17.8	0.03 ± 0.1	8.0 ± 2.2
0.01	−119.1 ± 22.0	41.9 ± 3.8	1.3 ± 0.3	186.4 ± 14.1	0.3 ± 0.1	11.5 ± 5.0
0.1	−110.7 ± 19.6	42.3 ± 4.5	1.4 ± 0.4	201.8 ± 25.9	0.2 ± 0.1	8.5 ± 2.3
1	−91.7 ± 17.5	42.3 ± 4.1	23.4 ± 1.4[b]	157.3 ± 9.7	0.8 ± 0.4	1.3 ± 1.6[a]
10	−117.7 ± 19.9	43.2 ± 5.0	31.8 ± 1.7[b]	175.3 ± 12.7	1.4 ± 0.5[b]	0.1 ± 1.8[a]

Values are means ± SE ($N = 6$ dogs). Results are expressed as micromoles per gram tissue wet weight per 60 minutes and are corrected for zero time.

A negative sign represents uptake of a metabolite whereas a positive sign depicts production. Abbreviation KG is alphaketoglutarate.

Significant difference from value obtained in the absence of 4-pentenoate in the flask: [a] $P < 0.05$; [b] $P < 0.01$

dehydrogenase and glutamate dehydrogenase near-equilibrium systems [17].

Statistical analysis

Unless otherwise specified, the term significant is used throughout the paper to describe a difference with a P value of less than 0.05. A variance analysis for repeated measurements on one level was performed, comparisons of all mean values being obtained by the Newman-Keuls procedure [18].

Results

Effects of 4-pentenoate on the metabolism of isolated renal cortical tubules incubated with various substrates

Glutamine. In the absence of 4-pentenoate, a significant fraction of the glutamine added to the medium was metabolically utilized, whereas glutamate, ammonia and glucose accumulated in the flasks (Table 1). The addition of 0.01 or 0.1 mM 4-pentenoate to the incubation medium did not change glutamine metabolism. However, concentration of 1 or 10 mM 4-pentenoate significantly accelerated the uptake of 1 mM glutamine (from 36 to 55 $\mu mol \cdot g^{-1} \cdot hr^{-1}$) and the production of ammonia and alphaketoglutarate. By contrast, the uptake of 5 mM glutamine and the production of ammonia were not increased by 4-pentenoate. In the presence of 10 mM 4-pentenoate, almost half of the alphaketoglutarate produced from glutamine metabolism was found as such in the flask instead of being further metabolized, suggesting a block at the alphaketoglutarate dehydrogenase level (Fig. 1). The fall in the production of alanine and aspartate with 5 mM glutamine (Fig. 2A) reflected the transaminations block induced by alphaketoglutarate accumulation. The transformation of alphaketoglutarate into pyruvate remained virtually unaffected by the addition of 4-pentenoate (Fig. 1). Lactate accumulation became quantitatively comparable to that of pyruvate instead of the normal 10 to 1 ratio observed in control conditions, indicating a reduction in cytoplasmic NADH. Gluconeogenesis was modestly increased by 0.1 mM 4-pentenoate, but markedly reduced by higher concentrations. The small amount of glutamine oxidation to CO_2 in control conditions was entirely suppressed by 4-pentenoate. The mitochondrial NAD+/NADH ratio calculated from the glutamate dehydrogenase system was markedly increased by 1 or 10 mM 4-pentenoate. Thus, the addition of 4-pentenoate led to a significant oxidation of the redox state of both cytosolic and mitochondrial compartments.

Lactate. In the absence of 4-pentenoate, a net utilization of lactate, pyruvate and aspartate as well as a net production of glutamate, ammonia, alanine and glucose were observed (Table 2). With 5 mM lactate, the higher concentration increased through an anaplerotic effect on the Krebs cycle, the alphaketoglutarate available for transformation into glutamate, therefore reducing the endogenous ammonia production.

The addition of 1 or 10 mM 4-pentenoate drastically reduced the utilization of lactate and pyruvate, the gluconeogenic flux and the apparent oxidation of these organic anions. The mitochondrial metabolism of pyruvate into oxaloacetate or acetyl CoA was thus drastically reduced by 4-pentenoate, suggesting a block at the pyruvate carboxylase and pyruvate dehydrogenase steps. With 5 mM lactate, 4-pentenoate increased ammonia production while reducing in a reciprocal fashion the production of alanine. The mitochondrial NAD+/NADH ratio was markedly increased by 1 or 10 mM 4-pentenoate (Fig. 3A). The cytosolic NAD+/NADH ratio calculated from the lactate dehydrogenase system was also increased by 1 or 10 mM 4-pentenoate (Fig. 3B).

When glutamine and lactate were placed together in the incubation medium, the changes induced by 4-pentenoate in the uptake or production of the various metabolites were in most instances similar to those observed either with glutamine alone (that is, increased glutamine uptake, and production of ammonia and malate) or with lactate alone (that is, reduced lactate uptake and gluconeogenesis). However with 5 mM concentrations of glutamine and lactate, the transamination of pyruvate into alanine was so important in the absence of 4-pentenoate (Fig. 2B) that no net GLDH deaminating flux was observed, possibly because pyruvate became the major source of reducing equivalents. This GLDH flux appeared with the addition of 4-pentenoate which decreased the availability of reducing equivalents.

Table 1. *Continued*

Alanine	Lactate	Pyruvate	Glucose
0.9 ± 0.5	0.4 ± 0.2	1.0 ± 0.2	10.1 ± 0.8
0.5 ± 0.5	0.6 ± 0.3	0.9 ± 0.1	9.9 ± 1.1
1.0 ± 0.5	0.4 ± 0.2	0.9 ± 0.2	12.0 ± 0.9[a]
2.9 ± 0.8[b]	11.5 ± 0.8[b]	8.4 ± 1.1[b]	3.8 ± 0.4[b]
2.2 ± 0.7[a]	9.3 ± 0.7[b]	8.3 ± 1.0[b]	3.4 ± 0.4[b]
10.7 ± 1.4	5.6 ± 0.8	4.5 ± 1.0	15.7 ± 1.2
10.7 ± 1.4	5.9 ± 0.8	4.4 ± 0.9	15.8 ± 1.3
9.9 ± 1.1	4.1 ± 0.8	4.2 ± 0.9	17.1 ± 1.3
4.6 ± 0.9[b]	11.8 ± 0.7[b]	9.0 ± 0.8[b]	3.7 ± 0.4[b]
3.4 ± 0.9[b]	9.3 ± 0.4[b]	7.9 ± 0.7[b]	3.2 ± 0.4[b]

Other substrates. With 1 mM glutamate in the medium, the addition of 10 mM 4-pentenoate accelerated glutamate uptake from 9 to 18 $\mu mol \cdot g^{-1} \cdot hr^{-1}$, induced an accumulation of alphaketoglutarate but did not significantly change ammonia production. With 5 mM glutamate in the medium, the addition of 4-pentenoate did not change significantly glutamate uptake and ammonia production but induced a marked accumulation of alphaketoglutarate. Four-pentenoate also increased the production of malate, lactate, pyruvate and alanine but reduced markedly gluconeogenesis from 12 to 0.4 $\mu mol \cdot g^{-1} \cdot hr^{-1}$.

When a concentration of 1 or 5 mM of either malate or glycerol was placed in the incubation medium, the addition of 4-pentenoate markedly reduced gluconeogenesis in a fashion comparable to that observed with other gluconeogenic substrates such as glutamine, glutamate and lactate: glucose production decreased from 12 to 0.5 $\mu mol \cdot g^{-1} \cdot hr^{-1}$ with 1 mM malate and from 23 to 6 $\mu mol \cdot g^{-1} \cdot hr^{-1}$ with 1 mM glycerol.

Effects of 4-pentenoate on the metabolism of isolated thick ascending limbs incubated with lactate

In the absence of 4-pentenoate and with lactate in the medium, a marked lactate uptake was observed (Table 3). Except for a modest increase in alanine production, no significant changes in the uptake or production of metabolites were induced by the addition of 0.01 or 0.1 mM 4-pentenoate to a suspension of thick ascending limbs incubated with lactate. However, findings apparently similar to those observed with cortical tubules were found with concentrations of 4-pentenoate of 1 or 10 mM. Four-pentenoate reduced markedly the net utilization of lactate (Fig. 4). The net pyruvate production was changed into a net pyruvate uptake (pyruvate was present initially at 1/10 of the lactate concentration), indicating a redox change. Indeed concomitant fall in pyruvate concentration and rise in lactate concentration, increasing markedly the lactate/pyruvate ratio and decreasing the cytoplasmic NAD+/NADH ratio, were observed. This redox shift contrasts with the oxidation observed with proximal tubules and indicates an accumulation of NADH, probably produced by the metabolism of 4-pentenoate itself. Indeed, both the mitochondrial and the cystolic NAD+/NADH ratios were decreased by the addition of 1 or 10 mM 4-pentenoate (Fig. 3). The lack of accumulation of pyruvate and alphaketoglutarate in the presence of 4-pentenoate in this tissue suggests the absence of a metabolic block at the alphaketoglutarate dehydrogenase and pyruvate dehydrogenase levels, in contrast to observations made with cortical tubules.

Effects of 4-pentenoate on the respiration of renal cortical tubules and thick ascending limbs

In renal cortical tubules, 4-pentenoate (1 mM) slightly increased the oxygen consumption from 384.7 to 403.8 μmol/g tissue wet wt/60 min. Thus the NADH availability for the respiratory chain was preserved following 4-pentenoate addition, in part through an accelerated conversion of glutamine into alphaketoglutarate. In thick ascending limbs, the oxygen consumption was also slightly increased by the addition of 1 mM 4-pentenoate (from 354.4 to 368.8 μmol/g tissue wet wt/60 min).

Effects of 4-pentenoate on the metabolism of ^{14}C-[1]-pyruvate or ^{14}C-[1]-alphaketoglutarate

Renal cortical tubules. The production of $^{14}CO_2$ from the metabolism of ^{14}C-[1]-pyruvate, measuring the rate of pyruvate oxidation to acetyl CoA, was linearly related to time and was reduced by 62% from 71.7 ± 3.7 to 27.6 ± 3.3 $\mu mol \cdot g^{-1} \cdot hr^{-1}$ by the addition of 4-pentenoate. Pyruvate uptake was simultaneously decreased from 89.3 ± 1.3 to 47.2 ± 4.8 $\mu mol \cdot g^{-1} \cdot hr^{-1}$ by 4-pentenoate. Glucose synthesis was immediately and markedly inhibited by 4-pentenoate. The lactate/pyruvate ratio in the flasks exceeded 6 in the absence of 4-pentenoate (without reaching the equilibrium value around 10), but was only 0.6 in the presence of 4-pentenoate which interfered with the production of NADH from pyruvate by suppressing the oxidation of pyruvate.

The production of $^{14}CO_2$ from the metabolism of ^{14}C-[1]-alphaketoglutarate, measuring the rate of alphaketoglutarate oxidation to succinyl CoA, was linearly related to time and was decreased (with alphaketoglutarate uptake) by 55% from 71.7 ± 2.5 to 32.6 ± 1.7 $\mu mol \cdot g^{-1} \cdot hr^{-1}$ by 4-pentenoate. Glucose production was again suppressed by 4-pentenoate. Lactate production was stimulated by 4-pentenoate, indicating the suppression of pyruvate oxidation.

Thick ascending limbs. The production of $^{14}CO_2$ from the metabolism of ^{14}C-[1]-pyruvate was linearly related to time (93.2 ± 11.9 $\mu mol \cdot g^{-1} \cdot hr^{-1}$) and was minimally affected by the addition of 4-pentenoate. Pyruvate utilization (133.4 ± 19.7 $\mu mol \cdot g^{-1} \cdot hr^{-1}$) was faster than in proximal tubules but was not changed by 4-pentenoate. No glucose synthesis was observed in this non-gluconeogenic nephron segment. The accumulation of lactate in the flask was unaffected by 4-pentenoate. Both in the absence or presence of 4-pentenoate, the lactate/pyruvate ratio remained around 1, a value much lower than the equilibrium value around 10. Therefore the oxidation of pyruvate and the production of NADH were not impaired by 4-pentenoate in this nephron segment.

The production of $^{14}CO_2$ from the metabolism of ^{14}C-[1]-alphaketoglutarate was minimal in thick ascending limbs (7.6 ± 2.3 $\mu mol \cdot g^{-1} \cdot hr^{-1}$). Alphaketoglutarate uptake and net lactate production also remained very small. The minimal metabolism of alphaketoglutarate in this nephron segment suggests a low permeability of the cell membrane to this organic anion.

Table 2. Effects of 4-pentenoate on the metabolism of renal cortical tubules incubated with lactate

4-Pentenoate concentration, mM	Glutamine	Glutamate	KG	Ammonia	Malate	Aspartate
Lactate 1 mM						
0	−0.1 ± 0.3	6.6 ± 1.3	0.5 ± 0.2	31.4 ± 3.8	−0.1 ± 0.1	−3.9 ± 1.2
0.01	−0.1 ± 0.3	6.8 ± 1.4	0.4 ± 0.2	33.4 ± 6.1	−0.1 ± 0.1	−3.8 ± 1.2
0.1	−0.2 ± 0.3	6.9 ± 2.0	0.5 ± 0.2	36.7 ± 5.8	−0.1 ± 0.1	−3.8 ± 1.5
1	−0.9 ± 0.3	−0.2 ± 1.2[b]	2.6 ± 0.9[a]	28.5 ± 4.8	0.2 ± 0.1[a]	−3.8 ± 1.4
10	−1.1 ± 0.3[a]	−1.0 ± 1.0[b]	3.2 ± 1.0[a]	28.0 ± 4.5	0.3 ± 0.1[b]	−3.6 ± 1.8
Lactate 5 mM						
0	−0.7 ± 0.3	13.5 ± 1.7	1.4 ± 0.3	11.5 ± 1.7	−0.1 ± 0.1	−4.8 ± 1.3
0.01	−0.6 ± 0.4	12.6 ± 1.5	1.3 ± 0.4	11.5 ± 1.8	0 ± 0.1	−4.8 ± 1.4
0.1	0.8 ± 2.0	11.6 ± 1.5	1.3 ± 0.4	10.8 ± 1.7	0.1 ± 0.1	−5.2 ± 1.2
1	−0.8 ± 0.3	−0.6 ± 1.1[b]	4.5 ± 0.9[a]	31.3 ± 5.3[b]	0.2 ± 0.1[a]	−4.8 ± 1.3
10	−1.2 ± 0.4	−1.1 ± 1.1[b]	4.8 ± 1.2[b]	27.2 ± 3.5[b]	0.5 ± 0.2[b]	−5.0 ± 1.4

Values are means ± SE (N = 6 dogs). Results are expressed as micromoles per gram tissue wet weight per 60 minutes and are corrected for zero time.

A negative sign represents uptake of a metabolite whereas a positive sign depicts production. Abbreviation KG is alphaketoglutarate.

Significant difference from value obtained in the absence of 4-pentenoate in the flask: [a] $P < 0.05$; [b] $P < 0.01$

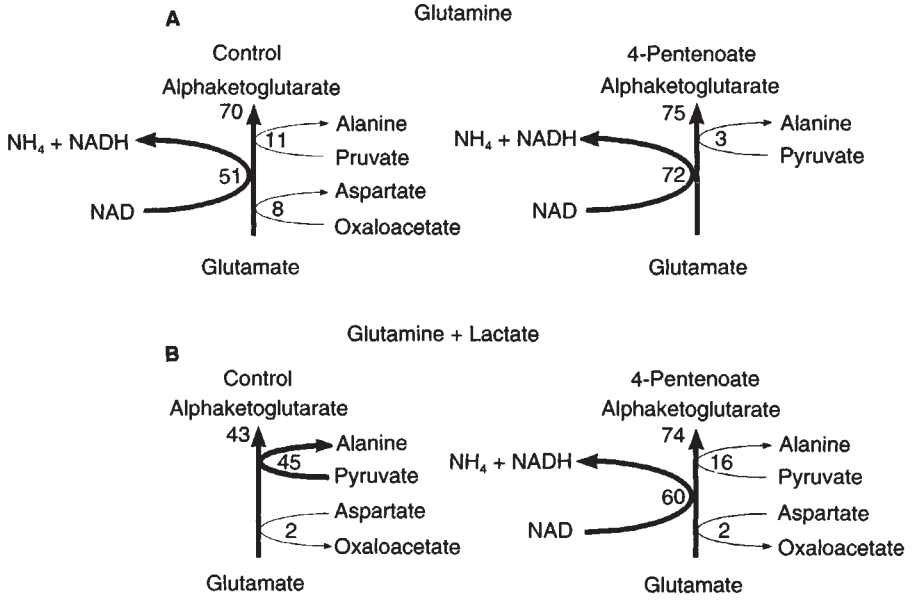

Fig. 2. *Metabolic fluxes (micromoles per gram tissue wet weight per 60 minutes) in renal cortical tubules of the three near-equilibrium reactions between glutamate and alphaketoglutarate catalyzed by glutamate dehydrogenase, alanine and aspartate aminotransferases with (left panel) or without 10 mM 4-pentenoate (right panel).* The upper panel represents the renal cortical tubules incubated with 5 mM glutamine, whereas these tubules incubated with 5 mM glutamine and 5 mM lactate are depicted in the lower panel.

Discussion

Effects of 4-pentenoate on glutamine metabolism in proximal tubules

The present study demonstrates that the addition of 1 or 10 mM 4-pentenoate to the incubation medium significantly increased the uptake of 1 mM glutamine and the production of ammonia by proximal cortical tubules in the dog, in a fashion comparable to that observed in vivo in the dog [1] and in rat renal cortical slices [10]. These findings were also reminiscent of those previously observed with valproate [19, 20] as well as with maleate [21, 22], a metabolic inhibitor known to deplete the mitochondrial CoA pool [23]. This accelerated ammoniagenesis contrasts with the decreased glutamine metabolism produced by short- or long-chain fatty acids [24] and resulted in alphaketoglutarate accumulation. Because the renal production of aspartate and alanine was not increased, the observed accumulation of alphaketoglutarate cannot be accounted for by an accelerated transamination, but probably resulted from an increased oxidative deamination of glutamate into alphaketoglutarate together with a block at the level of alphaketoglutarate dehydrogenase. This block was ascertained by the marked decrease by 4-pentenoate in the rate of $^{14}CO_2$ production from ^{14}C-[1]-alphaketoglutarate.

In fact, the data of the present study appear to be best accounted for by the following sequence of events in the 4-pentenoate-intoxicated mitochondria of proximal tubular cells. First, the metabolism of 4-pentenoate depleted mitochondrial free CoA [25] through its sequestration in inert and stable derivative(s) of 4-pentenoate [2, 3]. Second, the CoA-dependent mitochondrial oxidation of at least three substrates was impaired: long-chain fatty acids [4, 26], pyruvate [6] and alphaketoglutarate [8]. In our study, a block at the alphaketoglutarate

Table 2. *Continued*

Alanine	Lactate	Pyruvate	Glucose
3.1 ± 0.8	−61.2 ± 4.5	−4.6 ± 0.5	18.8 ± 1.3
3.9 ± 1.0	−62.1 ± 4.3	−4.5 ± 0.5	19.6 ± 1.5
5.0 ± 1.5	−60.0 ± 4.6	−4.3 ± 0.5	19.7 ± 1.5
6.7 ± 0.9[a]	−16.4 ± 3.7[b]	4.3 ± 1.4[b]	3.9 ± 0.5[b]
4.5 ± 0.8	−9.1 ± 3.6[b]	2.4 ± 1.4[b]	2.8 ± 0.4[b]
23.2 ± 1.6	−139.8 ± 15.3	−17.5 ± 1.5	30.6 ± 2.1
23.3 ± 1.5	−138.7 ± 16.0	−17.5 ± 1.4	29.9 ± 2.1
24.0 ± 1.6	−131.6 ± 16.0	−18.3 ± 0.9	27.3 ± 1.9
12.0 ± 1.2[b]	−48.1 ± 8.7[b]	4.0 ± 2.3[b]	5.7 ± 0.6[b]
8.6 ± 1.0[b]	−35.2 ± 10.4[b]	0 ± 2.3[b]	4.5 ± 0.3[b]

dehydrogenase level is suggested by: (1) the important amount of alphaketoglutarate produced by glutamine metabolism recovered as such in the flask instead of being further metabolized; (2) the decreased oxidation of ^{14}C-[1]-alphaketoglutarate. Third, this inhibition of mitochondrial metabolism decreased the supply of mitochondrial reducing equivalents, as reflected by the striking increase in the mitochondrial NAD+/NADH ratio observed in renal cortical tubules (Fig. 3B) and by the striking fall in lactate production when pyruvate is presented to the tubules. Only a modest rise was observed in vivo [1], probably because the predicted concentration of 4-pentenoate (before any metabolism) in the renal tissue was much less than 1 mM. Fourth, the oxidative deamination of glutamate into alphaketoglutarate and ammonia, an important residual source of NADH in these 4-pentenoate-intoxicated mitochondria, was accelerated. This increased deaminating GLDH flux occurred despite the marked accumulation of alphaketoglutarate, and must have resulted from the marked fall in mitochondrial NADH. This mechanism provided enough reducing equivalents to maintain a normal rate of respiration, that is, of the ATP turnover but not a normal lactate/pyruvate (NAD/NADH) ratio. Fifth, the resulting fall in intramitochondrial glutamate, a well-known inhibitor of the phosphate-dependent glutaminase [27], de-inhibited this enzyme and stimulated the deamidation of glutamine into glutamate and ammonia, and secondarily the entry of glutamine inside the mitochondria. This stimulation of glutamine metabolism was seen in the presence of 1 mM glutamine but was not observed with 5 mM glutamine, suggesting that in this condition enough alternative substrates were present to interfere with the toxic effect of 4-pentenoate.

Effects of 4-pentenoate on lactate and pyruvate metabolism

When renal cortical tubules and thick ascending limbs were incubated with lactate and pyruvate, 4-pentenoate markedly reduced the renal utilization of these two substrates in a fashion similar to that recently observed with valproate in these two preparations [19, 28]. This decreased oxidation of lactate and pyruvate might theoretically result from four mechanisms [29]: (1) an inhibited entry of lactate and pyruvate into renal tubular cells; (2) an increased cytoplasmic NADH decreasing lactate oxidation into pyruvate; (3) an inhibitory entry of pyruvate into mitochondria; and (4) an inhibition of pyruvate dehydrogenase activity. The latter was directly demonstrated by the marked fall in the rate of $^{14}CO_2$ production from ^{14}C-[1]-pyruvate in renal cortical tubules.

However, different mechanisms must account for the decreased utilization of lactate in renal cortical tubules and thick ascending limbs (Fig. 5). In renal cortical tubules (mostly proximal cells), the mitochondrial transformation of pyruvate into acetyl CoA became minimal in the presence of 4-pentenoate, suggesting a block at the level of pyruvate dehydrogenase. Thus the metabolism of 4-pentenoate in proximal cells interferes with the oxidation of pyruvate and limited the generation of reducing equivalents, presumably through CoA sequestration in 3-keto-4-pentenoyl CoA, an intermediate along the minor pathway of 4-pentenoate oxidation [3]. A mitochondrial CoA depletion resulting from the cellular metabolism of 4-pentenoate [2, 3] could indeed account for this inhibition. The secondary fall of acetyl CoA also explains the inhibition of pyruvate carboxylase and oxaloacetate production.

By contrast, in thick ascending limbs, the near-equilibrium oxidation of lactate into pyruvate was displaced in favor of lactate in the presence of 4-pentenoate. This inhibition probably resulted from the observed accumulation of reducing equivalents in both mitochondrial and (through the malate/aspartate shuttle) cytosolic compartments. Indeed, by contrast with the cortical tubules, 4-pentenoate did not alter the metabolic fluxes through pyruvate and alphaketoglutarate dehydrogenases ($^{14}CO_2$ production) and markedly increased the lactate/pyruvate ratio.

The renal metabolism of 4-pentenoate is thus different in cortical tubules and thick ascending limbs. Following its entry inside the mitochondria, 4-pentenoate can be metabolized by two different pathways [3]. The first two reactions are common to both pathways: activation of 4-pentenoate to 4-pentenoyl CoA which is oxidized to 2,4-pentadienoyl CoA. If enough NADPH is available, 2,4-pentadienoyl CoA is reduced through its major pathway to 3-pentenoyl CoA and is further metabolized into propionyl CoA and acetyl CoA. If NADPH is not available, 2,4-pentadienoyl CoA is hydrated through its minor pathway into 3-hydroxy-4-pentenoyl CoA which is oxidized to 3-keto-4-pentenoyl CoA, an inhibitory metabolite of fatty acid oxidation at the level of 3-ketoacyl-CoA thiolase [3].

Effects of 4-pentenoate on glucose metabolism

In the presence of lactate, glutamine, glutamate, malate or glycerol, the decreased glucose accumulation in the incubation medium indicates a decreased gluconeogenesis. Such a fall was also previously reported in isolated rat hepatocytes using pyruvate [9] and rat renal cortical slices utilizing various substrates [10, 11]. The lack of mitochondrial acetyl CoA, an obligatory allosteric regulator of pyruvate carboxylase [30], would inhibit the gluconeogenesis from lactate. The lack of cytosolic NADH (Fig. 3B) would decrease gluconeogenesis from glutamine and glutamate through inhibition of the glyceraldehyde-3-phosphate dehydrogenase [3, 5]. This may also account for the reduction of gluconeogenesis from malate. However this decreased gluconeogenesis cannot result only from the 4-pentenoate-induced inhibition of fatty acid oxidation and the decreased production of acetyl CoA and NADH in proximal tubules. Indeed, the marked inhibition by 4-pentenoate of gluconeogenesis from glycerol also suggests either a direct effect of 4-pentenoate or a

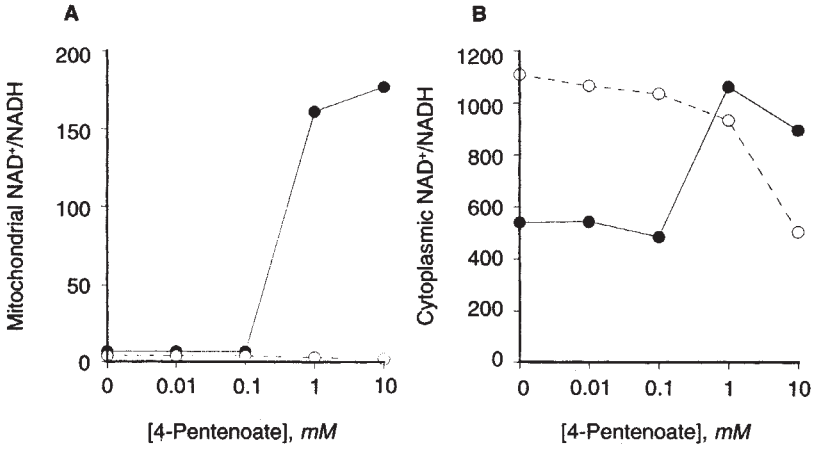

Fig. 3. *Mitochondrial (A) and cytoplasmic (B) NAD^+/NADH ratios as calculated respectively from the glutamate dehydrogenase and lactate dehydrogenase systems when renal cortical tubules (●—●) or thick ascending limbs (○----○) are incubated with 5 mM lactate and various concentrations of 4-pentenoate.*

Table 3. Effects of 4-pentenoate on the metabolism of thick ascending limbs incubated with lactate

4-Pentenoate concentration, mM	Lactate	Pyruvate	L/P ratio	Glutamate	KG	Ammonia	Aspartate	Alanine	Malate
Lactate 1 mM									
0	−71.9 ± 8.4	10.0 ± 1.9	3.2 ± 0.4	4.2 ± 0.9	1.5 ± 0.2	4.7 ± 0.8	−0.9 ± 0.7	3.7 ± 0.5	0.1 ± 0.1
0.01	−72.3 ± 7.6	9.8 ± 2.2	3.3 ± 0.4	5.1 ± 1.4	2.1 ± 0.3	5.3 ± 0.9	−0.8 ± 0.7	4.3 ± 0.7	0.1 ± 0.2
0.1	−64.7 ± 8.4	9.4 ± 1.6	3.4 ± 0.4	2.7 ± 1.4	1.6 ± 0.2	6.1 ± 1.3	−0.6 ± 0.8	5.2 ± 1.0[a]	0.2 ± 0.3
1	−59.6 ± 9.0[b]	5.8 ± 1.6	5.2 ± 0.9	2.7 ± 1.5	0.7 ± 0.1[a]	6.8 ± 1.1[a]	−0.3 ± 0.8[a]	4.2 ± 0.5	0.2 ± 0.2
10	−28.3 ± 7.5[b]	−7.7 ± 0.5[b]	18.8 ± 1.9[b]	0.8 ± 1.5[b]	0.9 ± 0.1	9.2 ± 1.4[b]	−1.2 ± 0.7	2.0 ± 0.5[b]	0.2 ± 0.1
Lactate 5 mM									
0	−112.5 ± 24.0	2.2 ± 6.9	8.6 ± 0.9	5.7 ± 1.1	2.8 ± 0.5	2.5 ± 0.6	−0.9 ± 0.6	6.1 ± 0.8	0.1 ± 0.2
0.01	−100.4 ± 27.1	0.3 ± 6.3	9.0 ± 0.9	5.8 ± 1.3	3.1 ± 0.4	3.2 ± 0.8	−1.0 ± 0.4	6.2 ± 0.9	0.1 ± 0.4
0.1	−91.0 ± 24.1[a]	0.2 ± 3.1	8.9 ± 0.6	5.1 ± 1.3	2.8 ± 0.2	2.8 ± 1.0	−1.1 ± 0.4	6.5 ± 1.1	0.1 ± 0.2
1	−94.0 ± 25.8	−7.7 ± 5.4	10.1 ± 0.9	4.1 ± 1.4	1.7 ± 0.3[b]	4.1 ± 0.8[a]	−0.7 ± 0.4	6.1 ± 1.0	−0.1 ± 0.5
10	−55.7 ± 22.9[b]	−33.2 ± 3.0[b]	18.4 ± 1.6[b]	4.4 ± 1.5	1.1 ± 0.2[b]	4.6 ± 1.1[b]	−1.0 ± 0.4	3.7 ± 0.7[b]	0.3 ± 0.1

Values are means ± SE (N = 6 dogs). Results are expressed as micromoles per gram tissue wet weight per 60 minutes and are corrected for zero time.

A negative sign represents uptake of a metabolite whereas a positive sign depicts production. Abbreviations are: KG, alphaketoglutarate; L/P ratio, concentration of lactate over that of pyruvate in micromoles per gram of tubules.

Significant difference from value obtained in the absence of 4-pentenoate in the flask; [a] $P < 0.05$; [b] $P < 0.01$

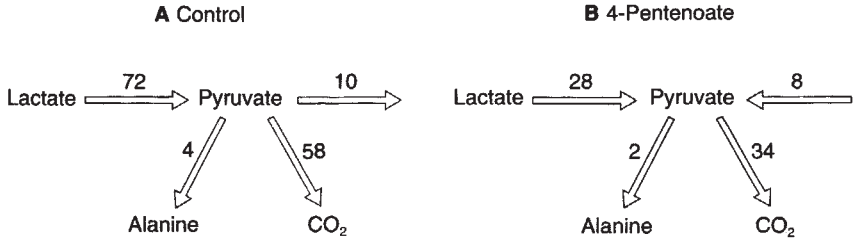

Fig. 4. *Renal metabolism of lactate 1 mM in thick ascending limbs in the absence (A) and in the presence of 10 mM 4-pentenoate (B).* Means for lactate and pyruvate extracted and alanine and pyruvate produced are given in Table 4 and these metabolic fluxes are expressed as micromoles per gram tissue wet weight per 60 minutes. Numbers close to each arrow represent mean fluxes calculated from measured values for reaction(s) represented by the arrow.

role of the intracellular ATP depletion induced by the inhibition of the Krebs cycle.

In summary, a striking difference was observed between the effects of 4-pentenoate in renal cortical tubules and thick ascending limbs. This discrepancy might be explained by the following hypothesis. In proximal tubules, the low hexokinase activity [12] might decrease the amount of glucose 6-phosphate available for the pentose phosphate pathway and the production of NADPH. Furthermore, the block at the level of alphaketoglutarate dehydrogenase would also decrease the malate available for its malic enzyme-dependent oxidation and the production of NADPH. Because the first reaction in the major pathway of the mitochondrial metabolism of 4-pentenoate is NADPH-dependent [3], the lack of NADPH might favor the minor pathway and the production of the inhibitory metabolite 3-keto-4-pentenoyl CoA. The mitochondrial CoA depletion would inhibit the CoA-dependent oxidation of fatty acids, pyruvate and alphaketoglutarate and the mitochondrial production of

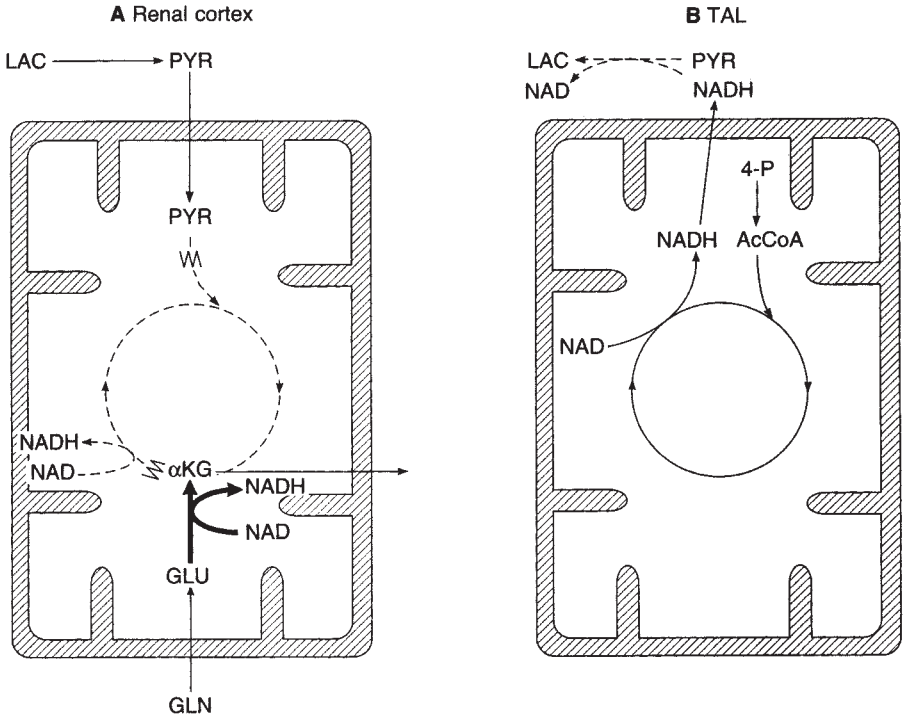

Fig. 5. *Cytoplasmic and mitochondrial effects of 4-pentenoate.* In the renal cortex, the depletion of mitochondrial reducing equivalents (resulting from the inhibition of pyruvate dehydrogenase and alphaketoglutarate dehydrogenase) stimulated the oxidative deamination of glutamate into alphaketoglutarate. In thick ascending limbs, the mitochondrial metabolism of 4-pentenoate increased the reducing equivalents in the mitochondrial and (through the malate/aspartate shuttle) cytosolic compartments. Abbreviations are: LAC, lactate; PYR, pyruvate; αKG, alphaketoglutarate; GLU, glutamate; GLN, glutamine; 4-P, 4-pentenoate; AcCoA, acetyl CoA.

NADH and NADPH and consequently increase the mitochondrial NAD+/NADH ratio.

In thick ascending limbs, the high hexokinase activity [12] might increase the glucose 6-phosphate availability for the pentose phosphate pathway or other pathway producing NADPH. This might have favored the major pathway of the mitochondrial metabolism of 4-pentenoate. Through this pathway, no inert CoA derivatives of 4-pentenoate are produced. The β-oxidation of 4-pentenoate would generate substantial amounts of NADH and therefore competitively decrease the oxidation of other substrates such as lactate and pyruvate.

However, additional experiments will be required to confirm this hypothesis and to elucidate the exact mechanisms responsible for the different effects of 4-pentenoate. These different findings in renal cortical tubules and thick ascending limbs underline the metabolic heterogeneity of these two nephron segments and emphasize the specific toxic effects of certain agents along the nephron. The decreased proximal reabsorption of various solutes observed in vivo and reproducing an experimental Fanconi's syndrome [1] probably results from this mostly proximal toxicity of 4-pentenoate.

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